HEWLETT® PACKARD

N-terminal Routine 3.1/3.5 Sequencer Methods

Technical Note

HP G1005A
N-terminal Protein Sequencing System

TN 96-1

The Routine 3.1/3.5 sequencer methods implement the Edman degradation chemistry (coupling, cleavage, and conversion) on Hewlett-Packard's column-based protein sequencer. These methods are appropriate for N-terminal amino acid sequence analysis of most protein and peptide samples in the nanomole to low picomole range. The methods are:

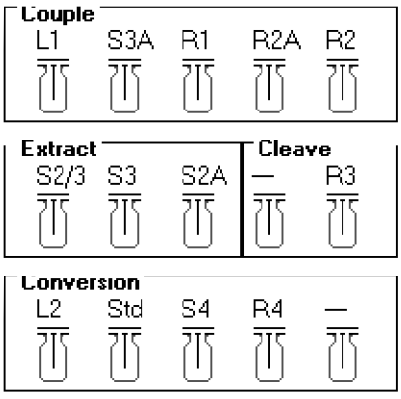
- Routine column
- Routine converter
- Routine Cycle 1 (column)
- Routine PTH Std (converter)
- Column Prep (column)

The Routine column and Routine converter methods typically control all sequence cycles with the exception of Cycle 1 for each sample. These methods are initiated by the Routine sequence program.

The method Routine Cycle 1 is used in the Routine sequence program as the column method for cycle 1. It provides a longer initial column drying time and performs a double coupling on the N-terminal amino acid. There is no specialized cycle 1 method for the converter.

The Routine PTH Std method delivers 10 pmol/100 µl PTH-amino acid standard solution to the on-line HPLC allowing the quantitation and identification of sequenced amino acids. You may choose to run a PTH-amino acid standard using Routine PTH Std when scheduling your sample.

The Column Prep method is used to wash the sequencer column prior to sample loading. This is especially important for low-level (<10 pmol) samples.



The Routine 3.1/3.5 sequencer methods require the bottle configuration shown. The reagents and solvents kits are purchased from Hewlett-Packard (HP G2046A for Routine 3.1 method or G2021A for Routine 3.5 method). These methods produce less than 1mL/cycle of liquid waste.

L1	neat acetonitrile
S3A	trifluoroacetic acid (TFA) in water (1:499)
R1	phenylisothiocyanate (PITC) in heptane (3:97)
R2A	octylamine in heptane (3:97)
R2	diisopropylethylamine (DIEA) in 1-propanol/water (1:3:6)
S2/3	acetonitrile/toluene (23:77)
S3	acetonitrile/toluene (15:85)
S2A	neat ethyl acetate
R3	neat trifluoroacetic acid (TFA)
L2	neat methanol
Std	mixture of PTH amino acids (10 pmol/100 µl) in acetonitrile with DPTU as a marker
S4	acetonitrile/water (10:90)
R4	trifluoroacetic acid (TFA) in water (1:3)

Method details

The steps in the Routine methods are described below. The steps indicate the ranges in volume that are appropriate for the various reagents/solvent deliveries. All sequencer methods are accessed by choosing the top menu item Edit/Method in the Protein Sequencer window. The sequencer column configuration used with Routine 3.1 and 3.5 methods consists of a RP sample column (top) mated with a strong anion exchange (SAX) column (bottom).

Routine 3.1 column method

Total time: 43.9 min (2631.6 sec)

All metering steps deliver the specified reagent to waste, not to the column.

Step 3: The delivery of R2A should completely fill the column (both halves).

Steps 6, 9: The delivery of R1 should completely fill the column (both halves).

Step 12: The volume of R2 delivered by the end of Step 12 should wet about 1/3 to 1/2 of the reverse-phase (RP) column. The bottom strong anion exchange (SAX) column should remain dry.

Step 13: No R2 should visibly wet the bottom SAX column.

Steps 14, 46: "Flush with L1" flushes the delivery lines and never wets the column.

Steps 20, 22, 24, 26, 35, 39: These solvent deliveries completely fill the column, followed by drying.

Step 30: During Step 30, no more than the upper frit of the top RP column should be wet with R3.

Step 33: After Step 33, the volume of S4 delivered to the converter should be 50 µl (±5µl).

Step 44: The delivery of S3A during Step 44 should at most wet the bottom 1/2 of the bottom SAX column. The top RP column remains dry.

Step 45: No S3A solvent should visibly wet the top RP column.

Step	Description	Time (sec)	Temp (°C)
1:	Couple: Dry column DOWN	40.0	55
2:	Couple: Meter R2A	5.2	55
3:	Couple: Deliver R2A DOWN (closed)	20.0	55
4:	Couple: Dry column DOWN	60.0	55
5:	Couple: Meter R1	5.2	55
6:	Couple: Deliver R1 DOWN (closed)	20.0	55
7:	Couple: Dry column DOWN	60.0	55
8:	Couple: Meter R1	5.2	55
9:	Couple: Deliver R1 DOWN (closed)	20.0	55
10:	Couple: Dry column DOWN	120.0	55
11:	Couple: Meter R2	11.7	55
12:	Couple: Deliver R2 DOWN	20.0	55
13:	Couple: Dry column DOWN	60.0	55
14:	Couple: Flush with L1	15.0	55
15:	Couple: React	200.0	55
16:	Couple: Deliver from coupling bank DOWN	60.0	55
17:	Couple: Dry column UP	120.0	55
18:	Wash: Purge solvent line		50
19:	Wash: Meter S2A	17.0	50
20:	Wash: Deliver and DRY DOWN (closed)	30.0	50
21:	Wash: Meter S2A	17.0	50
22:	Wash: Deliver and DRY DOWN (closed)	30.0	50
23:	Wash: Meter S2A	17.0	50
24:	Wash: Deliver and DRY DOWN (closed)	30.0	50
25:	Wash: Meter S2A	17.0	50
26:	Wash: Deliver and DRY DOWN (closed)	30.0	50
27:	Wash: Dry column DOWN	120.0	50
28:	Cleave: Purge cleavage line		50
29:	Cleave: Meter R3	2.8	50
30:	leave: Deliver R3 DOWN	30.0	50
31:	Cleave: React	300.0	50
32:	!Cleave: Dry column DOWN to converter	60.0	50
33:	!Extract: Deliver S4 to converter	7.0	50
34:	!Extract: Meter S2A	17.0	50
35:	!Extract: Deliver solvent DOWN (closed)	10.0	50
36:	!Extract: Dry column DOWN to converter	5.0	50
37:	!Extract: Evaporate converter	50.0	50
38:	!Extract: Meter S2A	17.0	50
39:	!Extract: Deliver solvent DOWN (closed)	10.0	50
40:	!Extract: Dry column DOWN to converter	5.0	50
41:	!Extract: Evaporate converter	50.0	50
42:	Extract: Dry column DOWN to waste	60.0	50
43:	Wash: Meter S3A	2.5	50
44:	Wash: Deliver S3A UP	10.0	50
45:	Wash: Dry column UP	30.0	50
46:	Wash: Flush with L1	15.0	55
47:	Wash: Dry column UP	120.0	55

! *indicates a system step*

Method (Column): Routine 3.1 Cycle 1

Total time: 58.7 min (3520.1 sec)

Step 1: Cycle 1 begins with an extended dry to remove any residual solvent from the sample loading process.

Step 3: The delivery of R2A should completely fill the column (both halves).

Steps 6, 9, 18: The delivery of R1 should completely fill the column (both halves).

Step 12, 21: The volume of R2 delivered by the end of Steps 12 and 21 should wet about 1/3 to 1/2 of the top RP column. The bottom SAX column should remain dry.

Steps 13, 22: No R2 should visibly wet the bottom SAX column.

Steps 23, 55: "Flush with L1" flushes the delivery lines and never wets the column.

Steps 29, 31, 33, 35, 44, 48: These solvent deliveries completely wet the column, followed by drying.

Step 39: During Step 39, no more than the upper frit of the top RP column should be wet with R3.

Step 42: After Step 42, the volume of S4 delivered to the converter should be 50 µl (±5µl).

Step 53: The delivery of S3A during Step 53 should at most wet the bottom 1/2 of the bottom SAX column. The top RP column remains dry.

Step 54: No S3A solvent should visibly wet the top RP column.

Step	Description	Time (sec)	Temp (°C)
1:	Couple: Dry column DOWN	300.0	55
2:	Couple: Meter R2A	5.2	55
3:	Couple: Deliver R2A DOWN (closed)	20.0	55
4:	Couple: Dry column DOWN	60.0	55
5:	Couple: Meter R1	5.2	55
6:	Couple: Deliver R1 DOWN (closed)	20.0	55
7:	Couple: Dry column DOWN	60.0	55
8:	Couple: Meter R1	5.2	55
9:	Couple: Deliver R1 DOWN (closed)	20.0	55
10:	Couple: Dry column DOWN	120.0	55
11:	Couple: Meter R2	11.7	55
12:	Couple: Deliver R2 DOWN	20.0	55
13:	Couple: Dry column DOWN	60.0	55
14:	Couple: React	200.0	55
15:	Couple: Deliver from coupling bank DOWN	60.0	55
16:	Couple: Dry column DOWN	60.0	55
17:	Couple: Meter R1	5.2	55
18:	Couple: Deliver R1 DOWN (closed)	20.0	55
19:	Couple: Dry column DOWN	60.0	55
20:	Couple: Meter R2	8.7	55
21:	Couple: Deliver R2 DOWN	20.0	55
22:	Couple: Dry column DOWN	60.0	55
23:	Couple: Flush with L1	15.0	55
24:	Couple: React	200.0	55
25:	Couple: Deliver from coupling bank DOWN	60.0	55
26:	Couple: Dry column UP	120.0	55
27:	Wash: Purge solvent line		50
28:	Wash: Meter S2A	17.0	50
29:	Wash: Deliver and DRY DOWN (closed)	30.0	50
30:	Wash: Meter S2A	17.0	50
31:	Wash: Deliver and DRY DOWN (closed)	30.0	50
32:	Wash: Meter S2A	17.0	50
33:	Wash: Deliver and DRY DOWN (closed)	30.0	50
34:	Wash: Meter S2A	17.0	50
35:	Wash: Deliver and DRY DOWN (closed)	30.0	50
36:	Wash: Dry column DOWN	120.0	50
37:	Cleave: Purge cleavage line		50
38:	Cleave: Meter R3	2.8	50
39:	Cleave: Deliver R3 DOWN	30.0	50
40:	Cleave: React	300.0	50
41:	!Cleave: Dry column DOWN to converter	60.0	50
42:	!Extract: Deliver S4 to converter	7.0	50
43:	!Extract: Meter S2A	17.0	50
44:	!Extract: Deliver solvent DOWN (closed)	10.0	50
45:	!Extract: Dry column DOWN to converter	5.0	50
46:	!Extract: Evaporate converter	50.0	50
47:	!Extract: Meter S2A	17.0	50
48:	!Extract: Deliver solvent DOWN (closed)	10.0	50
49:	!Extract: Dry column DOWN to converter	5.0	50
50:	!Extract: Evaporate converter	50.0	50
51:	Extract: Dry column DOWN to waste	60.0	50
52:	Wash: Meter S3A	2.5	50
53:	Wash: Deliver S3A UP	10.0	50
54:	Wash: Dry column UP	30.0	50
55:	Wash: Flush with L1	15.0	55
56:	Wash: Dry column UP	120.0	55

! *indicates a system step*

Method (Column):
Routine 3.5
Total time: 42.0 min (2518.0 sec)

Step 3: The delivery of R2A should completely fill the column (both halves).

Steps 6, 9: The delivery of R1 should completely fill the column (both halves).

Step 12: The volume of R2 delivered by the end of Step12 should visibly wet about 1/3 to 1/2 of the RP column. The bottom SAX column should remain dry.

Step 13: No R2 should visibly wet the bottom SAX column.

Steps 14, 42: "Flush with L1" flushes the delivery lines and never wets the column.

Steps 20, 22, 31, 35: These solvent deliveries completely fill the column, followed by drying.

Step 26: During Step 26, no more than the upper frit of the top RP column should be wet with R3.

Step 29: After Step 29, the volume of S4 delivered to the converter should be 50 µl (±5µl).

Step 40: The delivery of S3A during Step 40 should at most wet the bottom 1/2 of the bottom SAX column. The top RP column remains dry.

Step 41: No S3A solvent should visibly wet the top RP column.

Step	Description	Time (sec)	Temp (°C)
1:	Couple: Dry column DOWN	40.0	55
2:	Couple: Meter R2A	5.2	55
3:	Couple: Deliver R2A DOWN (closed)	20.0	55
4:	Couple: Dry column DOWN	60.0	55
5:	Couple: Meter R1	5.2	55
6:	Couple: Deliver R1 DOWN (closed)	20.0	55
7:	Couple: Dry column DOWN	60.0	55
8:	Couple: Meter R1	5.2	55
9:	Couple: Deliver R1 DOWN (closed)	20.0	55
10:	Couple: Dry column DOWN	120.0	55
11:	Couple: Meter R2	11.7	55
12:	Couple: Deliver R2 DOWN	20.0	55
13:	Couple: Dry column DOWN	60.0	55
14:	Couple: Flush with L1	15.0	55
15:	Couple: React	200.0	55
16:	Couple: Deliver from coupling bank DOWN	60.0	55
17:	Couple: Dry column UP	120.0	55
18:	Wash: Purge solvent line		50
19:	Wash: Meter S2/3	17.0	50
20:	Wash: Deliver and DRY DOWN (closed)	30.0	50
21:	Wash: Meter S2/3	17.0	50
22:	Wash: Deliver and DRY DOWN (closed)	30.0	50
23:	Wash: Dry column DOWN	120.0	50
24:	Cleave: Purge cleavage line		50
25:	Cleave: Meter R3	2.8	50
26:	Cleave: Deliver R3 DOWN with dry	30.0	50
27:	Cleave: React	300.0	50
28:	!Cleave: Dry column DOWN to converter	60.0	50
29:	!Extract: Deliver S4 to converter	7.0	50
30:	!Extract: Meter S3	17.0	50
31:	!Extract: Deliver solvent DOWN (closed)	10.0	50
32:	!Extract: Dry column DOWN to converter	5.0	50
33:	!Extract: Evaporate converter	50.0	50
34:	!Extract: Meter S2/3	17.0	50
35:	!Extract: Deliver solvent DOWN (closed)	10.0	50
36:	!Extract: Dry column DOWN to converter	5.0	50
37:	!Extract: Evaporate converter	50.0	50
38:	Extract: Dry column DOWN to waste	60.0	50
39:	Wash: Meter S3A	2.5	50
40:	Wash: Deliver S3A UP	10.0	50
41:	Wash: Dry column UP	30.0	50
42:	Wash: Flush with L1	15.0	55
43:	Wash: Dry column UP	120.0	55

! *indicates a system step*

Method (Column): Routine 3.5 Cycle 1

Total time: 60.4 min (3621.1 sec)

Step 1: Cycle 1 begins with an extended dry to remove any residual solvent form the loading process.

Step 3: The delivery of R2A should completely fill the column (both halves).

Steps 6, 9, 18: The delivery of R1 should completely fill the column (both halves).

Steps 12, 21: The volume of R2 delivered by the end of Steps 12 and 21 should wet about 1/3 to 1/2 column. The bottom SAX column should remain dry.

Steps 13, 22: No R2 should visibly wet the bottom SAX column.

Steps 23, 55: "Flush with L1" flushes the delivery lines and never wets the column.

Steps 29, 31, 33, 35, 44, 48: These solvent deliveries completely wet the column, followed by drying.

Step 39: During Step 39, no more than the upper frit of the top RP column should be wet with R3.

Step 42: After Step 42, the volume of S4 delivered to the converter should be 50 µl (±5µl).

Step 53: The delivery of S3A during Step 53 should at most wet the bottom 1/2 of the bottom SAX column. The top RP column remains dry.

Step 54: No S3A solvent should visibly wet the top RP column.

Step	Description	Time (sec)	Temp (°C)
1:	Couple: Dry column DOWN	300.0	55
2:	Couple: Meter R2A	5.2	55
3:	Couple: Deliver R2A DOWN (closed)	20.0	55
4:	Couple: Dry column DOWN	60.0	55
5:	Couple: Meter R1	5.2	55
6:	Couple: Deliver R1 DOWN (closed)	20.0	55
7:	Couple: Dry column DOWN	60.0	55
8:	Couple: Meter R1	5.2	55
9:	Couple: Deliver R1 DOWN (closed)	20.0	55
10:	Couple: Dry column DOWN	120.0	55
11:	Couple: Meter R2	11.7	55
12:	Couple: Deliver R2 DOWN	20.0	55
13:	Couple: Dry column DOWN	60.0	55
14:	Couple: React	200.0	55
15:	Couple: Deliver from coupling bank DOWN	60.0	55
16:	Couple: Dry column DOWN	120.0	55
17:	Couple: Meter R1	5.2	55
18:	Couple: Deliver R1 DOWN (closed)	20.0	55
19:	Couple: Dry column DOWN	60.0	55
20:	Couple: Meter R2	8.7	55
21:	Couple: Deliver R2 DOWN	20.0	55
22:	Couple: Dry column DOWN	60.0	55
23:	Couple: Flush with L1	15.0	55
24:	Couple: React	200.0	55
25:	Couple: Deliver from coupling bank DOWN	60.0	55
26:	Couple: Dry column UP	120.0	55
27:	Wash: Purge solvent line		50
28:	Wash: Meter S2/3	17.0	50
29:	Wash: Deliver and DRY DOWN (closed)	30.0	50
30:	Wash: Meter S2/3	17.0	50
31:	Wash: Deliver and DRY DOWN (closed)	30.0	50
32:	Wash: Meter S2/3	17.0	50
33:	Wash: Deliver and DRY DOWN (closed)	30.0	50
34:	Wash: Meter S2/3	17.0	50
35:	Wash: Deliver and DRY DOWN (closed)	30.0	50
36:	Wash: Dry column DOWN	120.0	50
37:	Cleave: Purge cleavage line		50
38:	Cleave: Meter R3	2.8	50
39:	Cleave: Deliver R3 DOWN with dry	30.0	50
40:	Cleave: React	300.0	50
41:	!Cleave: Dry column DOWN to converter	60.0	50
42:	!Extract: Deliver S4 to converter	7.0	50
43:	!Extract: Meter S3	17.0	50
44:	!Extract: Deliver solvent DOWN (closed)	10.0	50
45:	!Extract: Dry column DOWN to converter	5.0	50
46:	!Extract: Evaporate converter	50.0	50
47:	!Extract: Meter S2/3	17.0	50
48:	!Extract: Deliver solvent DOWN (closed)	10.0	50
49:	!Extract: Dry column DOWN to converter	5.0	50
50:	!Extract: Evaporate converter	50.0	50
51:	Extract: Dry column DOWN to waste	60.0	50
52:	Wash: Meter S3A	2.5	50
53:	Wash: Deliver S3A UP	10.0	50
54:	Wash: Dry column UP	30.0	50
55:	Wash: Flush with L1	15.0	55
56:	Wash: Dry column UP	120.0	55

! *indicates a system step*

Method (converter) Routine 3.1/3.5

Total time: 33.1min (1988.1sec)

Step 1: During Step 1, the liquid in the converter partially evaporates but must not dry down completely (10-20 µl remains).

Step 2: During Step 2, the volume of R4 delivered to the converter should be approximately 70 µl (±5µl).

TEST Delivery protocol:
To check the delivery volume of R4, copy Step 2 to the clipboard and run from the clipboard. At the end of Step 2, remove the con version flask from the heating chamber. Carefully unscrew the flask and measure the delivered volume.

Step 4: By the end of Step 4, the converter will have been dry for 100-200 secs.

Step 5: By the end of Step 5, 100-120 µl of L2 will have been delivered to the converter.

Step 6: By the end of Step 6, the converter will have been dry for 75-100 secs.

Step 7: After Step 7, the volume of S4 delivered to the converter should be 75-80 µl.

TEST Delivery protocol:
To check the delivery volume of S4, copy steps 7, 8, and 9 to the clipboard and run from the clip board. At the end of Step 9, remove the conversion flask from the heating chamber. Carefully unscrew the flask and measure the delivered volume

Step 10: Before injection the injector loop should be filled, leaving solvent visible in both the inlet and outlet lines of the injector loop.

Step	Description	Time (sec)	Temp (°C)
1:	Convert: Evaporate	80.0	70
2:	Convert: Deliver R4	29.0	70
3:	Convert: React	400.0	70
4:	Convert: Evaporate dry	350.0	80
5:	Convert: Flush with L2	15.0	80
6:	Convert: Evaporate dry L2	140.0	70
7:	Convert: Deliver S4 dripwise	15.5	60
8:	Convert: Mix and equilibrate	15.0	60
9:	Convert: Solubilize	20.0	60
10:	Convert: Fill loop and inject	30.0	60
11:	Convert: Empty converter	30.0	45
12:	Convert: Deliver R4 wash	30.0	45
13:	Convert: Empty converter	30.0	45
14:	Convert: Flush with L2	85.0	45
15:	Convert: Mix	20.0	45
16:	Convert: Empty converter	30.0	45
17:	Convert: Dry waste line	100.0	45
18:	Convert: Dry vent line	100.0	45

Method (Converter): Routine 3.1/3.5 PTH Std

Total time: 34.3 min (2060.0 sec)

This Routine PTH-standard method is appropriate with any of the sequence programs: 3.1, 3.1 PVDF, or 3.5. Ten (10) pmols of PTH standard are delivered to the conversion flask in this method.

Step 7: Step 7 should add 100 µl (±2 µl) of PTH-standard to the dry converter.

TEST Delivery protocol:
To check the delivery volume of PTH-standard, copy Step 7 to the clipboard and run from the clip board. At the end of Step 7, remove the conversion flask from the heating chamber. Carefully unscrew the flask and measure the delivered volume.

Step 8: The solvent is completely evaporated by the end of the 180 sec dry.

Step 9: After Step 9, the volume of S4 delivered to the converter should be 75-80 µl.

Step 12: Before injection the injector loop should be filled, leaving solvent visible in both the inlet and outlet lines of the injector loop.

Step	Description	Time (sec)	Temp (°C)
1:	Convert: Empty converter	60.0	60
2:	Convert: Flush with L2	85.0	60
3:	Convert: Mix	20.0	60
4:	Convert: Empty converter	30.0	60
5:	Convert: Dry waste line	200.0	60
6:	Convert: Dry vent line	200.0	60
7:	Convert: Deliver PTH Std with L2 purge	6.9	60
8:	Convert: Evaporate dry standard	180.0	60
9:	Convert: Deliver S4 dripwise	15.5	60
10:	Convert: Mix and equilibrate	15.0	60
11:	Convert: Solubilize	20.0	60
12:	Convert: Fill loop and inject	30.0	60
13:	Convert: Empty converter	60.0	45
14:	Convert: Deliver R4	25.0	45
15:	Convert: Empty converter	30.0	45
16:	Convert: Flush with L2	170.0	45
17:	Convert: Mix	20.0	45
18:	Convert: Empty converter	30.0	45
19:	Convert: Dry waste line	200.0	45
20:	Convert: Dry vent line	200.0	45

Method (Column): Column Prep 3.1/3.5

Total time: 21.0 min (1262.3 sec)

Steps 3, 14, 20, 23, 26, 28, 30, 32: These solvent deliveries completely fill the column, followed by drying.

Steps 6, 17, 27: These reagent deliveries should completely fill the column (both halves).

Steps 9: The delivery of R2 in Step 9 should completely fill the column (both halves).

Step	Description	Time (sec)	Temp (°C)
1:	Col prep: Purge coupling line	8.0	60
2:	Col prep: Meter L1	12.0	60
3:	Col prep: Deliver from coupling bank DOWN	15.0	60
4:	Col prep: Dry column DOWN	10.0	60
5:	Col prep: Meter R2A	10.0	60
6:	Col prep: Deliver R2A DOWN (closed)	20.0	60
7:	Col prep: Dry column DOWN	30.0	60
8:	Col prep: Meter R2	50.0	60
9:	Col prep: Deliver R2 DOWN (closed)	45.0	60
10:	Col prep: wait	90.0	60
11:	Col prep: Dry column DOWN to waste	60.0	60
12:	Col prep: Purge coupling line	30.0	60
13:	Col prep: Meter L1	12.0	60
14:	Col prep: Deliver from coupling bank DOWN	15.0	60
15:	Col prep: Dry column DOWN	120.0	60
16:	Col prep: Meter R3	15.0	60
17:	Col prep: Deliver from cleavage bank DOWN		60
18:	Col prep: Dry column DOWN	10.0	60
19:	Col prep: Meter S3A	25.0	60
20:	Col prep: Deliver from coupling bank DOWN	40.0	60
21:	Col prep: Dry column DOWN	10.0	60
22:	Col prep: Meter L1	12.0	60
23:	Col prep: Deliver from coupling bank DOWN	15.0	60
24:	Col prep: Dry column DOWN	60.0	60
25:	Col prep: Meter S2A	17.0	60
26:	Col prep: Deliver and DRY DOWN (closed)	30.0	60
27:	Col prep: Meter S2A	17.0	60
28:	Col prep: Deliver and DRY DOWN (closed)	30.0	60
29:	Col prep: Meter S2A	17.0	60
30:	Col prep: Deliver and DRY DOWN (closed)	30.0	60
31:	Col prep: Meter S2A	17.0	60
32:	Col prep: Deliver and DRY DOWN (closed)	30.0	60
33:	Col prep: Dry column DOWN	60.0	60

These sequencer programs are recommended for use after prolonged sequencer shut-down to remove residual chemicals deposited in the sequencer flow path and converter. It is also recommended for use prior to low picomole level analysis following analysis at high picomole sample loads. Install an empty sequencer column assembly (HP G2286A) in the column position to be cleaned and schedule one cycle of the column/ converter clean program at this position.

Method (Column): Column Line Wash

Total time: 11.5 min (688.5 sec)

Step	Description	Time (sec)	Temp (°C)
1:	Wash: Purge coupling line	8.0	50
2:	Wash: Meter L1	12.0	50
3:	Wash: Deliver from coupling bank DOWN	15.0	50
4:	Wash: Dry column DOWN	60.0	50
5:	Wash: Meter R3	15.0	50
6:	Wash: Deliver from cleavage bank DOWN		50
7:	Wash: Dry column DOWN	10.0	50
8:	Wash: Meter S3A	25.0	50
9:	Wash: Deliver from coupling bank DOWN	40.0	50
10:	Wash: Dry column DOWN	10.0	50
11:	!Wash: Deliver L1 DOWN	100.0	50
12:	!Wash: Wait	100.0	50
13:	!Wash: Dry column DOWN	30.0	50
14:	!Wash: Dry column UP to converter	20.0	50
15:	Wash: Dry column DOWN	20.0	50
16:	Wash: Purge solvent line	20.0	50
17:	Wash: Dry column DOWN	60.0	50

Method (Converter): Converter wash

Total time: 21.4 min (1285.5 sec)

Step	Description	Time (sec)	Temp (°C)
1:	Convert: Flush with L2	80.0	80
2:	Convert: Empty converter	40.0	80
3:	Convert: Purge L2 line to waste	30.0	80
4:	Convert: Deliver R4	180.0	80
5:	Convert: Deliver S4	240.0	80
6:	Convert: Mix	30.0	80
7:	Convert: Empty converter	40.0	80
8:	Convert: Flush with L2	180.0	80
9:	Convert: Empty converter	40.0	80
10:	Convert: Dry waste line	100.0	80
11:	Convert: Dry vent line	100.0	80

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**Printed in the U.S.A. July 1996
(23) 5965-3467E**